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Monoenyl hydrocarbons in female body wax of the yellow peach moth
as synergists of aldehyde pheromone components

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Abstract

The non-polar components of female body wax and pheromone gland extracts of the yellow peach moth synergistically enhanced male behavioral responses from close to pheromone sources in wind tunnel tests when mixed with an aldehyde pheromone blend. When the non-polar fractions of female body wax were further separated by column chromatography, synergistic activities were found in the 3% and 50% ether in hexane fractions, and they additively increased male responses. The main components of the first fraction were (Z)-9-tricosene, (Z)-9-pentacosene, (Z)-9-heptacosene, (Z)-9-nonacosene and (Z)-9-hentriconten. Only (Z)-9-heptacosene showed a significant synergistic effect in enhancing male responses, but the other components had no effect. A mixture of the five monoenyl hydrocarbons lost activity at lower doses than 5 ng. Natural ratios of these hydrocarbons in the female body wax and pheromone gland extracts were similar, but amount of (Z)-9-heptacosene in the female body wax was significantly higher than in the pheromone gland extracts. We conclude that (Z)-9-heptacosene increase male responses to aldehyde pheromones, and unknown component(s) in the 50% ether in hexane fraction are required for full synergistic enhancement by the non-polar fractions of the female body wax and the pheromone gland extracts.

Keywords: Monoenyl hydrocarbons; body wax; yellow peach moth; pheromone

41 synergists

Introduction

The yellow peach moth, *Conogethes punctiferalis* (GUENÉE), seriously damages a number of crops, fruits and vegetables in Tropical Asia, Eastern Asia and Australian regions (Sekiguchi, 1974; Waterhouse, 1993). A binary mixture of (*E*)-10-hexadecenal (E10-16: Ald) and (*Z*)-10-hexadecenal (Z10-16: Ald) at a ratio of 90:10 was reported as an effective pheromone lure in field tests (Konno et al., 1982). However low performance of lures baited with the mixture was later experienced in monitoring and mating disruption in some regions of Japan (Kondo et al., 2008). Therefore, the possibilities of inconsistencies in ratios of natural pheromone components of the previous lures and shortage of unknown additional component(s) were predicted. This led to the re-analysis of the natural ratio of these two aldehyde components and it was revised to 95.4: 4.5, which never improved performance of the new lure (Kimura, 2002; Lin and Honda, unpublished data). Furthermore, EAD active compounds (hexadecanal and (*E*)-10-hexadecenol) subsequently identified in the pheromone gland extract also did not improve the activity of previously identified aldehyde blend (Kimura, 2002).

In our previous report, we showed that non-polar components of the pheromone gland extracts and female body wax extracts synergistically enhanced male behavioral

responses during the final stages of response to the pheromone sources when mixed with the aldehyde pheromone blend (Xiao and Honda, 2010).

In the present paper, we report the identification of monoenyl hydrocarbons as enhancing factors in the female body wax extracts as well as the pheromone gland extracts, and discuss their function in the pheromone system of the yellow peach moth.

Materials and methods

Insects

The larvae of the yellow peach moth were reared on an artificial diet and chestnuts or corn (Honda et al., 1979). Adults were sexed at the pupal stage and kept separately in cages at $25 \pm 1^\circ\text{C}$, 40-60% R.H. and under a 15 L-9 D photoperiod. Adults were provided with a 10% sugar solution from cotton pads. A 15 W red incandescent lamp was used for observations during scotophase.

Extracts and fractionation

Abdominal distal segments including the sex pheromone glands were excised with micro-scissors from 2 to 4-day-old virgin female moths anesthetized with CO_2 at 5-8 hr after lights-off. Abdominal tips were extracted for 15 min at room temperature with redistilled *n*-hexane (ca. 1 μl per insect) containing 0.01% butylated hydroxyl toluene (Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) as an anti-oxidant. The pooled pheromone gland extracts were collected in screw vials (200 female equivalents per vial) with Teflon^R-lined caps and stored at -20°C until use. Two to 4 day old virgin female moths and male moths were anesthetized with CO_2 during photophase (4-6 hr after light on) to avoid contamination of the sex pheromone aldehydes (Konno et al., 1982), and then their whole bodies were extracted with ca. 0.5 ml per insect of

redistilled hexane for 2 min in a small glass vial. Female body wax extracts and male body wax extracts were passed through a cotton filter in a Pasteur pipette^R to remove body scales, respectively, and the extracts of 200 insects were pooled in each vial, and stored at -20°C.

Pheromone gland extracts, female and male body wax extracts were also subjected to open column chromatography (1 cm in diameter, 3 cm in length) loaded with 1 g of Florisil (60-100 µm particle size, Wako Pure Chemical Industries Co. Ltd., Osaka, Japan) impregnated with 7% distilled water (Carroll, 1961), to obtain non-polar fractions (NPFs) with elution of 10 ml hexane. The NPFs were further fractionated by column chromatography with an open column (1 cm diameter, 3 cm length) packed with 1 g silica gel (Wako gel C-200, particle size: 75 -150 µm) impregnated with 10% AgNO₃ (99.8%, Wako). Each NPF was successively eluted with 10 ml each of hexane (Fr. 1), 1% (Fr. 2), 3% (Fr. 3), 10% (Fr. 4), 30% (Fr. 5) and 50% ether in hexane (Fr. 6), and 10 ml ether (Fr. 7). The fractions were concentrated to 1 FE (female equivalent) /µl under a N₂ stream and stored at -20°C until use.

Bioassay

All bioassays were conducted in a transparent acrylic wind tunnel (2 m in length, 0.3 m in diameter), in which rectified wind was provided at 30 cm/s by an electric fan located

at the downwind outlet. Male behavioral responses to stimuli were observed at $25 \pm 1^\circ\text{C}$ and 40-60% RH under illumination with a red incandescent light at ca. 2 lux. One μl of test materials dissolved in hexane were loaded on a triangular filter paper (0.5 cm in base, 1.5 cm in height), and the filter papers were hung 15 cm high from the floor and 15 cm from the upwind end of the wind tunnel. Prior to tests, the streams of stimulus plumes in the wind tunnel were simulated with TiCl_4 . Newly emerged 2 to 4 day old males were individually transferred into small metal mesh cages (6 cm in diameter, 6 cm in height) at least 1 hr before testing. The cage was hung on a ring-holder at 15 cm from the ceiling and 15 cm from the downwind end of the wind tunnel. Male moths were allowed to naturally leave the cage just after setting a stimulus source at the upwind end, and the following four behaviors were recorded for 3 min: (1) starting flight, (2) catching plume (male moth flies upwind along the plume), (3) close to source (hovering around within 10 cm of the bait) and (4) source contact (Xiao and Honda, 2010; Mazor and Dunkelblum, 1992). The duration of remaining close to the source and number of source contact was also recorded. After each test, the inside wall of the wind tunnel was rinsed well with 70% ethanol and then completely ventilated.

Dimethyl disulfide (DMDS) treatment

The fraction (s) which was active in the wind tunnel bioassays were derivatized to DMDS adducts to determine the position of the double bonds in the compounds (Francis and Veland, 1981). DMDS (200 μ l, Wako) and 50 μ l of I₂ solution (60 μ g/ μ l,) in diethyl ether were added to the fractions. The mixtures were sealed in glass ampoules and stirred for 24 h at room temperature and then extracted three times with 1 ml hexane after addition of 5% Na₂S₂O₃ solution. The extracts were subsequently dehydrated with anhydrous Na₂SO₄ and concentrated for GC/MS analysis.

Chemicals

E10-16: Ald and Z10-16: Ald were obtained from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan), and a blend of these two aldehydes (ratio of 95.5: 4.5) was prepared after purification of both isomers (99.5% in purity) by chromatography over 10% AgNO₃-SiO₂. (Z)-9-Tricosene (Z9-23: HC) and (Z)-9-nonacosene (Z9-29: HC) were given by S. Matsuyama. (Z)-9-Pentacosene (Z9-25: HC), (Z)-9-heptacosene (Z9-27: HC) and (Z)-9-hentricontene (Z9-31: HC) were synthesized by Z-selective Wittig reactions of corresponding aldehydes with C₉-ylide, generated by mixing potassium hexamethyldisilazane (KHMDs) and nonyltriphenylphosphonium bromide. As an

example, synthesis of Z9-25: HC is described below.

A solution of hexadecanal (0.37 g, obtained by oxidation of 1-hexadecanol with PCC) in THF was added to a cooled (-30°C) stirred suspension of nonylidenetriphenylphosphorane, generated by KHMDs and 2.14 g of corresponding phosphonium bromide in THF/HMPA (3: 1). The reaction mixture was held overnight with stirring, allowed to warm to room temperature, then poured into an aqueous NH_4Cl_2 solution and extracted with ether. The combined ether layers were washed with brine, dried over MgSO_4 and the solution was evaporated. The resulting crude material was once chromatographed over SiO_2 (eluent: hexane) and subsequently purified with a Sephadex^R LH-20 (Pharmacia Fine Chemicals, particle size: 25-100 μm) column (eluent: CHCl_3) to produce 0.22 g of (Z)-9-heptacosene (99% in purity).

Chemical analysis

Gas chromatography (GC) analyses were carried out on a Shimadzu GC-17A gas chromatograph equipped with an FID on an HP-5 column (30 m \times 0.32 mm ID, film thickness 0.25 μm , Agilent Technologies, USA), using a temperature program of 190°C to 280°C at $10^{\circ}\text{C}/\text{min}$, with an initial 2 min hold at 190°C and a final 10 min hold at

280°C. Samples were injected in splitless mode at 280°C using He as the carrier gas (24 cm/sec). The temperature of the detector was 280°C.

Gas chromatography-mass spectrometry analyses were conducted with a mass selective detector (5972 Series, Hewlett-Packard) coupled with a HP 5890 Series II GC equipped with a HP-5MS column (30 m × 0.25 mm ID, film thickness 0.25 µm, Agilent Technologies, USA). The oven temperature was held for 2 min at 140°C and increased to 280°C at 5°C /min, and held at 280°C for 10 min. Samples were injected in splitless mode at 280°C using He as the carrier gas (37 cm/sec). The temperature of the interface was maintained at 280°C.

For quantification of monounsaturated hydrocarbons in body wax extracts and crude pheromone extracts, a tetracosane was used as an external standard for Z9-23: HC and Z9-25: HC, and an octacosane for Z9-27: HC, Z9-29: HC and Z9-31: HC.

Statistical analysis

Data on starting flight, catching plume and close to source were first analyzed by χ^2 Fisher's exact probability test using the actual number of insects. When probability was significant ($p < 0.05$), multiple comparisons were performed employing Ryan's method. Data on mean time for duration of close to source and mean number of

176 source contacts by male moths were analyzed by Tukey's test after ANOVA. Software
177 package R, version 2.10.0 (R Development Core Team, 2009), was used for statistical
178 analyses.
179

Results

Enhancing activity by female body wax hydrocarbon fractions

As reported by Xiao and Honda (2010), a non-polar fraction of female body wax extracts of the yellow peach moth enhanced male responses to a pheromone source in the final stage of mating behavior when mixed with an aldehyde blend consisting of E10-16: Ald and Z10-16: Ald in a ratio of 95.4: 4.5. To survey the active components, the non-polar fraction was further separated into seven fractions, Fr. 1 –Fr. 7, by AgNO₃-impregnated silica gel column chromatography and the synergistic activity was evaluated in wind tunnel tests (Table 1). The male response rate in the first three stages, starting flight, catching plume and close to source, never increased even when each of the seven fractions was tested as a binary mixture with the aldehyde blend. However, the mean time remaining close to source and the mean number of source contacts by male moths remarkably increased with Frs. 3, 5 and 6 when mixed with the aldehyde blend. No significant increase in these responses was observed in the other four fractions.

The synergistic effects of these seven fractions on male response to the pheromone source were further evaluated by mixing the seven fractions in different combinations (Table 2). In two mixtures with combinations of Frs. 3 and 5, and Frs. 5 and 6, significantly higher activities than the aldehyde blend as a control were observed in the

mean time remaining close to source and the mean number of source contacts, but the activity was not significantly different between the two combinations. The highest enhancing effects were observed with a binary mixture of Frs. 3 and 6, a ternary mixture of Frs. 3, 5 and 6 and a mixture of all seven fractions. However, the enhancing activities of these three mixtures were not significantly different in the mean time remaining close to source and the mean number of source contacts by male moths.

Chemical analysis of hydrocarbon synergists

The combination of Frs. 3 and 6 enhanced the activity of the aldehyde blend the same as the mixture of all fractions, indicating the synergistic compounds exist in these two fractions. The Fr. 3 was first analyzed and the results are reported in the present paper. The major peaks (percent of total peak area: $91.61 \pm 0.83\%$) in the gas chromatogram profile of the Fr. 3 showed five homologous compounds. Their retention times in GC were 19.81 min, 23.05 min, 26.10 min, 29.05 min and 31.74 min, respectively. GC/MS analysis of these compounds gave a common base peak at m/z 97 (100%), and molecular ions at m/z 322 (12%), 350 (10%), 378 (9%), 406 (8%) and 434 (8%), respectively, supposing these compounds to be tricosene, pentacosene, heptacosene, nonacosene and hentriacontene, respectively. After DMDS treatment, all corresponding adducts showed a same fragment ion at m/z 173, indicative of a double

bond located at the 9-position in the molecules. Additionally, in the GC/MS chromatogram, each peak of pentacosene, heptacosene and nonacosene was closely followed by a peak with a much smaller area, which formed three pairs of peaks. Moreover the two peaks in each pair gave the same mass spectra. These paired peaks were estimated to represent the geometrical isomers. By comparison of GC retention times and mass spectra between the natural compounds and authentic chemicals, the earlier peaks in each paired peaks were confirmed to be the Z isomers. Thus, the dominant monounsaturated hydrocarbons were identified as Z9-23: HC, Z9-25: HC, Z9-27: HC, Z9-29: HC and Z9-31: HC.

Not only NPF from the female body wax, but also NPFs from the pheromone gland extracts and male body wax extracts gave the same monoenyl hydrocarbons in the Fr. 3 after AgNO₃-impregnated silica gel column chromatography. Moreover, the female body wax extracts and pheromone gland extracts showed similar compositions of those hydrocarbons, which were much different from those in male body wax extracts. Additionally, the total amount of those hydrocarbons in the female body wax was about 25 times and 33 times higher than in the pheromone gland extracts and male body wax extracts, respectively (Table 3).

Bioassay of monounsaturated hydrocarbons

Male moth responses in the last two behavioral stages, mean time remaining close to

source and mean number of source contacts, to the mixtures of five homologous monoeryl hydrocarbons from Z9-23: HC to Z9-31: HC and the aldehyde blend are shown in Fig. 1. The highest male responses were observed to Z9-27: HC, and increases and decreases in the number of carbons atoms in the molecule resulted in diminution of the male response to the mixture of monoeryl hydrocarbons and the aldehyde blend. The responses evoked by Z9-27: HC were as high as that of the all monoeryl hydrocarbons mixture, with the addition of the aldehyde blend, respectively. The synergistic effect of all monoeryl hydrocarbons mixture disappeared when Z9-27: HC was removed. The activities with Z9-27: HC and the mixture of all monoeryl hydrocarbons also corresponded to those of the Fr. 3 that was separated by AgNO₃-impregnated silica gel column chromatography from the NPF of female body wax extracts (Table 1).

The dose-response relationship of synergism with the mixture of all monoenes with the aldehyde blend at different doses is shown in Fig. 2. A similar dose-response relationship was found between the mean time remaining close to source and the mean number of source contacts. A remarkable increase in male responses was observed when the doses were 50 ng and 300 ng. However, at a dose of 5 ng and below, no significant increase in responses was observed.

Discussion

We reported synergism of the NPF of the pheromone gland extracts and female body wax extracts with aldehyde pheromone components (Xiao and Honda, 2010). To identify the active components, the NPF of female body wax extracts was separated into several fractions based on their degree of unsaturation. As shown in Table 1, when the fractions were mixed with the aldehyde blend, significant increases in responses were observed in Frs. 3, 5 and 6. This suggests that different types of unsaturated hydrocarbons in these fractions synergistically contribute to the increase in male responses during the last two behavioral stages of mating. Moreover, the activities of only the binary mixture of Frs. 3 and 6 was as high as the three-fraction mixture and NPF, indicating that components contributing to the activity in the NPF were mainly contained in the Frs. 3 and 6 (Table 2).

In the Fr. 3 of the female body wax extracts, the total amount (per insect) of the main monoaryl hydrocarbons was ca. 25 times higher than in the pheromone gland extracts, but the relative ratios showed no difference between the two sources (Table 3). Therefore, synergistic pheromonal activities of the hydrocarbons with the aldehydes in the yellow peach moth probably come from female body wax hydrocarbons. Additionally, the same hydrocarbons with a quite different relative ratio and a much

smaller total amount than female body wax extracts were also found in male body wax extracts, which contained ca. 7.4 ng/male equivalent of Z9-27: HC (Table 3). This amount was between those of Z9-27: HC in mixture of all monoenes at the dose of 5 ng which showed no synergism with the aldehyde blend, and the dose of 50 ng which showed the synergism (Fig. 2). Such amount of Z9-27: HC in male body wax was either inadequate to elicit the synergism with the aldehyde blend, or the synergism was covered by unknown factors, which resulted in no synergism found in male body wax extracts (Xiao and Honda, 2010).

Most lepidopteran pheromones are known to consist of multi-components that are classified into the Type I group including alcohols, acetates and aldehydes, the Type II group with unsaturated hydrocarbons or their epoxy derivatives, and miscellaneous pheromone components that are classified as neither Type I nor Type II (Ando et al., 2004). Meanwhile, both Type I and Type II compounds are also used together as the sex pheromones of several species (Hill and Roelofs, 1981; Hill et al., 1982; Cabrera et al., 2001; Leal et al., 2005; Millar et al., 2005; Gibb et al., 2007). Compounds in both the Type I and Type II groups of lepidopteran sex pheromones have been identified in pheromone extracts obtained by extracting the female's abdominal tips containing the sex pheromone glands.

292 On the other hand, it is well known that some monounsaturated hydrocarbons
293 originating from the body wax serve as contact sex pheromones in Diptera, Coleoptera,
294 and Hymenoptera (Uebel et al., 1975; Zhang et al., 2003; Mant et al., 2005; Ginzl et al.,
295 2006; Böröczky et al., 2009). However, in Lepidoptera, the pheromonal activity of
296 cuticular monoenyl hydrocarbons has rarely been studied. In the swallowtail
297 butterfly *Papilio polytes*, (Z)-7-tricosene, (Z)-7-pentacosene and (Z)-7-heptacosene were
298 identified in body waxes of both male and female adults. However, the pheromonal
299 activity of these compounds has not been investigated (Omura and Honda, 2005).

300 In the yellow peach moth, Z9-23: HC, Z9-25: HC, Z9-27: HC, Z9-29: HC and Z9-31:
301 HC were identified from female body wax extracts as well as pheromone gland extracts,
302 and at least Z9-27: HC was confirmed to have synergism with the aldehyde pheromone
303 components, E10-16: Ald and Z10-16: Ald. A similar phenomenon may also occur
304 widely in lepidopteran species. Especially in species that use both Type I and Type II
305 components as sex pheromones, unsaturated hydrocarbons (Type II) may be produced
306 not only in the pheromone gland but also from the body surface.

307 Increased activity was observed when each of the monoenyl hydrocarbons (tested in 50
308 ng) was mixed with the aldehyde blend. However, a remarkable increase was found in
309 the mixture of Z9-27: HC and the aldehyde blend when compared with the aldehyde

blend alone. Moreover, a mixture of all monoenyl hydrocarbons (total amount: 50 ng) including Z9-27: HC and the remaining four monoenes also elicited synergism as high as Z9-27: HC (Fig. 1). Together with the fact that the actual amount of Z9-27: HC in the mixture of all monoenes was less than 50 ng, these results suggest that the monoenes other than Z9-27: HC may also contribute to synergism in this species.

A mixture of monoenes was tested for a dose-response relationship and the dose range of synergism was greater than 5 ng (Fig. 2). Considering that the natural amount of those monoene hydrocarbons in body wax extracts is ca. 900 ng per one female (Table 3), we can calculate that the synergistic activity of monoene hydrocarbons with an aldehyde blend may be lost after a 180 fold dilution of one female equivalent. A 1,000 fold dilution of 1 female equivalent of NPF from female body wax extracts showed synergism in a previous report (Xiao and Honda, 2010). These results are not contradictory because the synergism of NPF from female body wax extracts actually represents a total synergism of active compounds in both Frs. 3 and 6.

As a summary, in the yellow peach moth, monoenyl hydrocarbons, at least Z9-27: HC, in female body waxes synergistically enhanced the response of male moths to the synthetic sex pheromone, the aldehyde blend. However, such synergism is not high enough unless other synergistic compounds in the Fr. 6 of NPF from female body wax

extracts are added. Furthermore, identification of the synergistic monoenyl hydrocarbons as well as other unsaturated hydrocarbons, probably polyenyl hydrocarbons in the Fr. 6, will undoubtedly improve the understanding of the complete sex pheromone system of the yellow peach moth. At present the identification and synthesis of the compounds in the Fr. 6 are underway.

The pheromonal activity of cuticular monounsaturated hydrocarbons was demonstrated in laboratory bioassays, but field tests are also needed for confirmation. The findings of this study are expected to improve field performance of sex pheromone lures for the yellow peach moth.

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Figure legends

Fig. 1 Activities of mixtures of synthesized monoenyl hydrocarbons and the aldehyde blend as mean time remaining close to source (open columns) and mean number of source contacts (closed columns) in wind tunnel tests. Values are shown as the mean \pm standard error. Numbers in parentheses are replications of different individual insects. For the mixtures, 10 ng of the aldehyde blend was mixed with either 50 ng monoenes or 50 ng of a mixture in which all monoenes were mixed at the natural ratio. Values accompanied by the same letter were not significantly different by Tukey's test after ANOVA at $p < 0.05$.

Fig. 2 Dose-response relationship of the synergism of the synthetic monoenyl hydrocarbons mixture to the aldehyde blend in the mean time remaining close to source (open columns) and mean number of source contacts (closed columns) in wind tunnel tests. Values are shown as the mean \pm standard error. Numbers in parentheses are replications of different individual insects. For the mixtures, 10 ng of the aldehyde blend was mixed with each dose of a mixture in which all monoenes were mixed at the natural ratio. Values accompanied by the same letter were not significantly different by Tukey's test after ANOVA at $p < 0.05$.

Tables and figures

Table 1 Synergistic activity in wind tunnel tests with an aldehyde blend of fractions separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extracts of *C. punctiferalis*.

| Treatment ^{a,b} | N | Male responses (%) ^d | | | Mean time | Mean number |
|--------------------------|----|---------------------------------|----------|----------|------------------------------|----------------------|
| | | Starting | Catching | Close to | remaining close | of source |
| | | flight | plume | source | to source (sec) ^e | contact ^e |
| Fr. 1 | 16 | 100.0 a | 100.0 a | 87.5 a | 26.7± 3.0 e | 6.9± 1.1 c |
| Fr. 2 | 16 | 93.8 a | 93.8 a | 93.8 a | 34.8± 3.5 e | 11.3± 1.3 c |
| Fr. 3 | 20 | 100.0 a | 100.0 a | 100.0 a | 56.0± 5.6 cd | 23.8± 2.7 b |
| Fr. 4 | 16 | 93.8 a | 93.8 a | 87.5 a | 37.8± 4.5 de | 12.7± 1.8 c |
| Fr. 5 | 15 | 100.0 a | 100.0 a | 93.3 a | 61.7± 10.6 bc | 24.2± 3.9 b |
| Fr. 6 | 17 | 94.1 a | 94.1 a | 94.1 a | 79.4± 7.2 b | 31.2± 2.9 b |
| Fr. 7 | 13 | 100.0 a | 92.3 a | 92.3 a | 25.6± 5.9 e | 4.1± 1.4 c |
| NPF(FBW) ^c | 17 | 100.0 a | 100.0 a | 100.0 a | 100.3± 7.8 a | 43.7± 3.3 a |
| Control | 27 | 100.0 a | 88.9 a | 88.9 a | 23.5± 2.2 e | 8.3± 1.0 c |

^aTen ng (1 female equivalent, FE) of the mixture of E10-16: Ald and Z10-16: Ald at a ratio of 95.5: 4.5 was added to all treatments of 1 FE.

^b Fr. 1 (100% hexane fraction), Fr. 2- 6 (1%, 3%, 10%, 30% and 50% ether in hexane fractions) and Fr. 7 (100% ether fraction) separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extracts.

446 ^c One FE of the non-polar fraction of female body wax extracts in Florisil column
447 chromatography.

448 ^dData with different letters in the same column are significantly different at $p < 0.05$ by
449 Ryan's multiple comparisons after Fisher's exact probability test ($P < 0.05$).

450 ^eData with different letters in the same column are significantly different at $p < 0.05$ by
451 Tukey's multiple comparisons after ANOVA ($P < 0.05$).

452

Table 2 Synergistic activity in wind tunnel tests of an aldehyde blend of mixed fractions separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extracts of *C. punctiferalis*.

| Treatment ^{a, b} | N | Male responses (%) ^d | | | Mean time | Mean number |
|--|----|---------------------------------|----------------|-----------------|--|--------------------------------|
| | | Starting flight | Catching plume | Close to source | remaining close to source (sec) ^e | of source contact ^e |
| Fr. 3+ Fr. 5 | 24 | 87.5 a | 87.5 a | 87.5 a | 53.3± 6.3 b | 20.4± 3.0 b |
| Fr. 5+ Fr. 6 | 20 | 90.0 a | 90.0 a | 90.0 a | 64.0± 7.0 b | 26.1± 3.4 b |
| Fr. 3+ Fr. 6 | 16 | 100.0 a | 100.0 a | 100.0 a | 112.8± 5.6 a | 48.1± 2.6 a |
| Fr. 3 + Fr. 5+ Fr. 6 | 16 | 100.0 a | 100.0 a | 93.8 a | 117.6± 9.8 a | 52.7± 5.0 a |
| Fr. 1+ Fr. 2+ Fr. 4+ Fr. 7 | 18 | 100.0 a | 83.3 a | 83.3 a | 24.0± 2.7 e | 3.0± 1.2 c |
| Recombined ^c fractions mixture | 15 | 100.0 a | 100.0 a | 100.0 a | 108.0± 6.4 a | 47.3± 3.2 a |
| Control | 28 | 92.9 a | 92.9 a | 89.3a | 28.6± 2.4 c | 8.6± 1.9 c |

^aTen ng (1 female equivalent, FE) of the mixture of E10-16: Ald and Z10-16: Ald at a ratio of 95.5: 4.5 was added to all treatments of 1 FE.

^b Fr. 1 (100% hexane fraction), Fr. 2- 6 (1%, 3%, 10%, 30% and 50% ether in hexane fractions) and Fr. 7 (100% ether fraction) separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extracts.

^c A mixture of 1 FE each of Fr. 1- Fr. 7 separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extract were tested.

463 ^dData with different letters in the same column are significantly different at $p < 0.05$ by

464 Ryan's multiple comparisons after Fisher's exact probability test ($P < 0.05$).

465 ^eData with different letters in the same column are significantly different at $p < 0.05$ by

466 Tukey's multiple comparisons after ANOVA ($P < 0.05$).

467

Table 3 The percentage of each (Z)-9-monoene area in all dominant (Z)-9-monoenes areas in the GC profile and the amount of each (Z)-9-monoenes in the 3% ether in hexane fraction separated by AgNO₃-silica gel chromatography from the non-polar fraction of female body wax extracts, pheromone gland extracts and male body wax extracts of *C. punctiferalis*.

| Monoenes | Ratio (%) ^a | | | Amount (ng) ^{a, b} | | |
|-----------|------------------------|-----------------|------------------|-----------------------------|-----------------|------------------|
| | FBW ^c | PG ^d | MBW ^e | FBW ^c | PG ^d | MBW ^e |
| Z9-23: HC | 2.0± 0.1 | 3.7± 0.4 | 14.0± 0.3 | 16.2± 1.1 | 1.4± 0.1 | 3.2± 0.2 |
| Z9-25: HC | 7.5± 0.1 | 7.9± 0.2 | 24.3± 0.2 | 50.0± 4.3 | 2.4± 0.2 | 5.1± 0.4 |
| Z9-27: HC | 29.6± 0.1 | 27.2± 0.4 | 24.9± 0.5 | 272.0± 27.3 | 10.3± 0.8 | 7.4± 0.7 |
| Z9-29: HC | 51.5± 0.7 | 50.7± 0.6 | 26.4± 0.8 | 470.3± 48.7 | 18.7± 1.5 | 7.8± 0.8 |
| Z9-31: HC | 9.5± 0.1 | 10.6± 0.4 | 10.4± 0.5 | 90.5± 10.0 | 4.3± 0.3 | 3.5± 0.3 |
| Total | - | - | - | 899.0± 91.3 | 37.0± 2.8 | 27.0± 2.2 |

^a Values are shown as the mean± standard deviation.

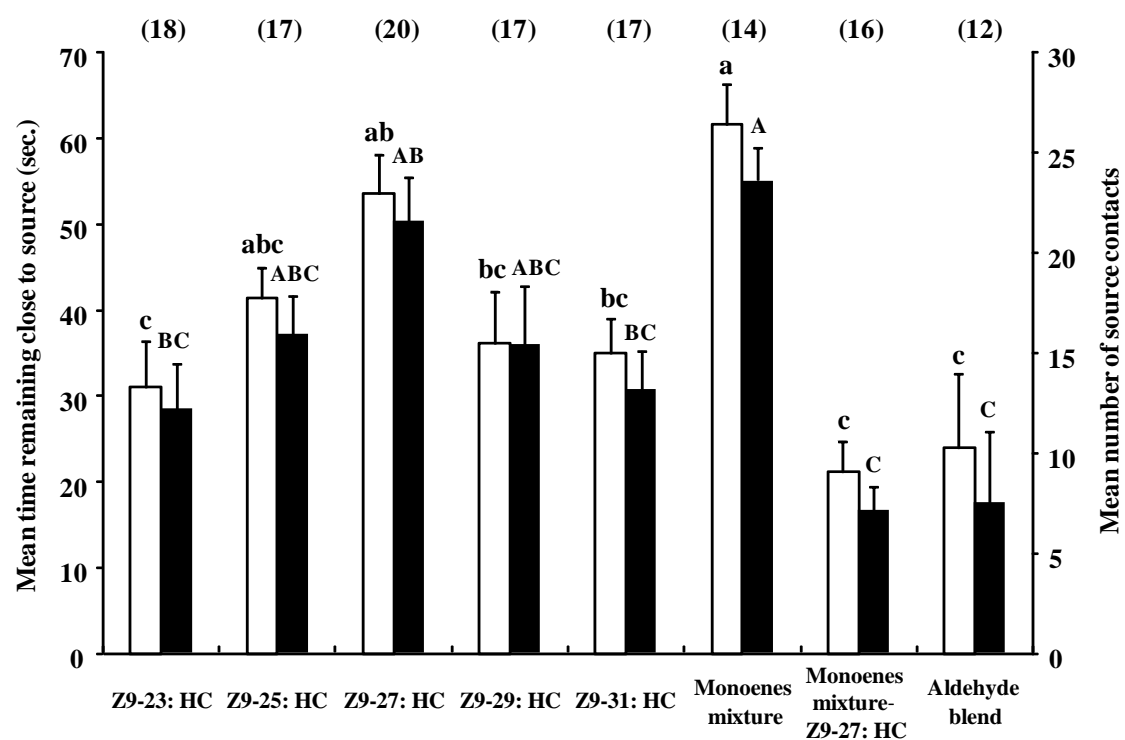
^b Monoenyl hydrocarbons in one female equivalent of female body wax extracts and pheromone gland extracts, and one male equivalent of male body wax extracts were calculated.

^c FBW: Female body wax extracts.

478 ^dPG: Pheromone gland extracts.

479 ^eMBW: Male body wax extracts.

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Mixture of monoaryl hydrocarbon (s) and the aldehyde blend

Fig. 1

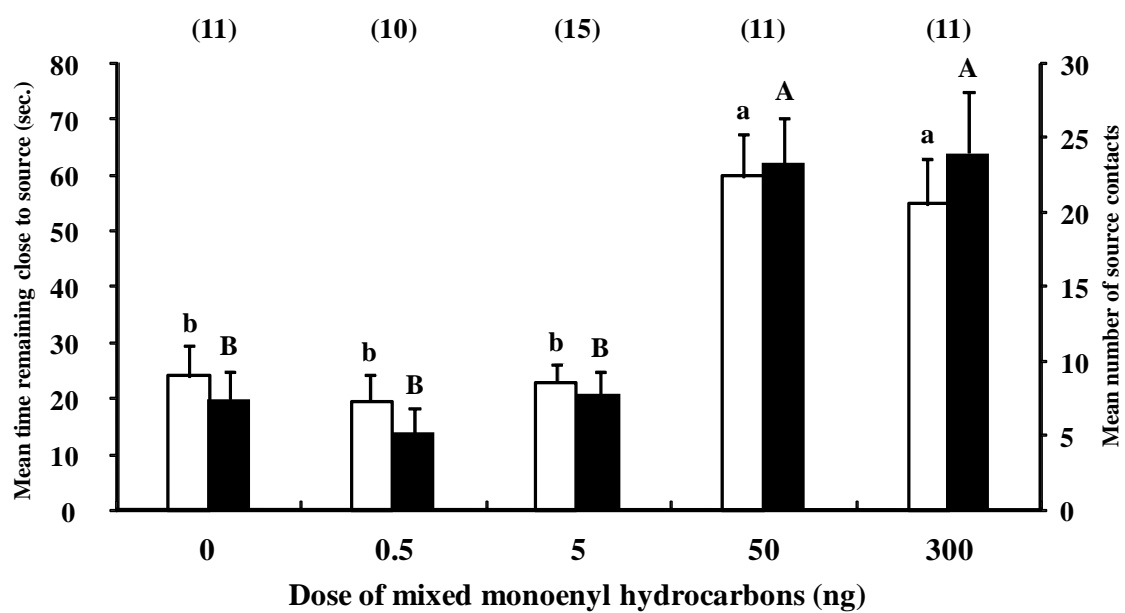


Fig. 2